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The K153R variant in the myostatin gene and sarcopenia at the end of the human lifespan

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Abstract We studied the A55T, E164K, I225T, K153R and P198A variants in the myostatin (*GDF8*) gene, muscle strength and mass, and physical function during daily living in 41 nonagenarians [33 women, age range, 90, 97]. No participant carried a mutant allele of the aforementioned variants, except three participants (all women), who carried the R allele of the K153R polymorphism, with one of them (woman aged 96 years) being homozygous. Overall, in KR women muscle phenotype values (1RM leg press and estimated muscle mass) were low-to-normal compared to the whole group (~25th–50th percentile), and their functional capacity (Barthel and Tinetti tests) was normal. In the woman bearing the RR genotype, values of muscle mass and functional capacity were below the 25th percentile. She is the first RR Caucasian whose phenotype has been characterised specifically. In summary, heterozygosity for the *GDF8* K153R polymorphism does not seem to exert a negative influence on the muscle phenotypes of women who are at the end of the human lifespan, yet homozygosity might do so. More research on larger cohorts of nonagenarians is needed to corroborate the present findings.

Keywords Activities of daily living · GDF-8 · Muscle strength · Nonagenarians

Marta González-Freire and Gabriel Rodríguez-Romo contributed equally.

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Introduction

The number of people approaching the theoretical endpoint of the human lifespan is increasing. In elderly people, functional capacity is directly dependent on muscular fitness as these persons typically experience age-associated declines in skeletal muscle mass and strength, i.e., sarcopenia (Brooks and Faulkner 1994; van Dijk et al. 2005). Sarcopenia contributes to the decreased capacity for independent living and reduced ability to cope with activities of

daily life (ADLs), increasing the burden for the caregiver and community (Serra Rexach et al. 2009). To identify the factors that influence muscle fitness at the end of the human lifespan is of clinical and public health interest. Genetics is one of these factors. There are candidate genes that may explain individual variations in muscle fitness, such as unfavourable genotypes potentially associated with accelerated sarcopenia.

The myostatin (*MSTN* or growth differentiation factor 8, *GDF8* [MIM601788]) gene is receiving growing attention (Huygens et al. 2004). It encodes myostatin, a skeletal muscle-specific secreted peptide that functions to limit muscle growth (McPherron et al. 1997). In transgenic animal models, prolonged absence of myostatin reduces sarcopenia (Siriett et al. 2006). Systemic treatment with myostatin inhibitors provides an adequate safety margin for clinical studies (Wagner et al. 2008). Whereas Wagner et al. (2008) did not show improvements in muscle strength or function, they observed that a few individuals had an increased muscle size (as measured with dual-energy radiographic absorptiometry and muscle histology), supporting the bioactivity of myostatin inhibitors.

Several polymorphisms and mutations have been identified in the *GDF8* gene with diverse functional consequences. Of the identified polymorphisms, the Lys(K)153Arg(R) variation located in exon 2 (rs1805086, 2379 A>G replacement) is a candidate to influence skeletal muscle phenotypes. The frequency of the mutant R allele is of about 3–4% among Caucasians, with a frequency of mutant homozygotes (RR) below 1% (Corsi et al. 2002; Ferrell et al. 1999; Kostek et al. 2009). Such low allelic frequency certainly limits the possibility of studying large groups of people carrying the R variant. Though controversy exists with regards to adults (Kostek et al. 2009), the K153R polymorphism is associated with muscle strength, with the infrequent mutant R allele possibly exerting a negative influence in old (yet <80 years) Caucasian people (Ferrell et al. 1999; Huygens et al. 2004; Seibert et al. 2001).

It was the purpose of this study to assess the association of the *GDF8* K153R polymorphism with muscle phenotypes and functional capacity during ADLs in Spanish (Caucasian) nonagenarians. We also genotyped the *GDF8* variants A55T (rs1805065, located in exon 1), and E164K (rs35781413), I225T and P198A (all located in exon 2).

Materials and methods

A total of 41 (n=33 women) nonagenarians (age range, 90–97 years) were recruited from a geriatric nursing home (*Los Nogales*-Pacífico, Madrid, Spain) and received a comprehensive medical examination. Inclusion criteria were: age ≥90 years, able to ambulate with or without assistance, able to communicate and being capable and willing to provide consent. Exclusion criteria were acute or terminal illness, myocardial infarction in the past 3 months, unstable cardiovascular disease, upper or lower extremity fracture in the past 3 months, severe dementia and presence of neuromuscular disease or drugs affecting neuromuscular function. The Medical Ethics Committee of *Hospital General Universitario Gregorio Marañón* (Madrid, Spain) approved the study and all participants provided written informed consent.

We assessed dynamic muscular strength of the lower body by estimating one repetition maximum (1RM) seated leg press (Technogym, Barcelona, Spain) (Brzycki 1993; Serra Rexach et al. 2009). Initial loads were 70–100% of body weight. Following a brief rest period, increments of 2–4 kg were added until maximal effort was achieved for each lift, usually after 5 trials or less. All participants were able to lift the initial load at least one time. Participants were instructed on proper breathing and lifting form for each movement (Serra Rexach et al. 2009). We estimated whole-body muscle mass as detailed elsewhere (Lee et al. 2000).

We assessed participants' gait and balance abilities using the *Tinetti scale* (Tinetti 1986). For gait evaluation, the subject stands with the examiner, walks across the room, first at "usual" pace, then back at "rapid, but safe" pace (utilising usual walking aids) and the following tasks are scored (0 to 2, indicating highest to lowest level of impairment respectively): initiation of gait, step length and height, step symmetry, step continuity, path, trunk sway and walking stance. For balance evaluation, the subject is seated in a hard, armless chair and the following manoeuvres are tested (score, 0, 1 or 2): sitting balance, arises, attempts to arise, immediate standing balance (first 5 s), standing balance, 'nudged', eyes

closed, turning 360° and sitting down. The maximum sum-score of both gait and balance components is 28 points. Patients who score below 24 are at risk for falls, and the risk of falling is high with a score below 19. The validity of this test for screening old adults at risk for falling is well established (Raiche et al. 2000).

We also determined the participant's ability to perform ADLs independently with the Barthel score (Mahoney and Barthel 1965). The *Barthel index* is a valid instrument that is widely used to measure the capacity of a person for the execution of ten basic activities in daily life, obtaining a quantitative estimation of the individual's level of independency (Collin et al. 1988; Mahoney and Barthel 1965). The ten items include eating, transferring from bed to chair, using the toilet, bathing/showering, personal hygiene (tooth brushing, shaving) dressing, walking, stair climbing and bowel and bladder control. Each individual item is scored with 0 (unable to perform without complete help), 5 (able to perform the activity with little help) or 10 (able to perform without any help). The sum-score ranges from 0 (*totally dependent*) to 100 (*totally independent*).

Sequences corresponding to the A55T, E164K, I225T, K153R and P198A variants were amplified by the polymerase chain reaction (PCR) during early fall 2009 (for E164K, I225T, K153R and P198A) and late January 2010 (for A55T) in the Genetics Laboratory of the *Universidad Europea de Madrid*. The primers used for the A55T variant were: 5′-CAAGTTGTCTCTCAGACTG-3′ and 5′-CAACAGTCAGCAGAACTGT-3′. The primers used for the other variants were 5′-GAAAACCCAAATGTTGCTTC-3′ and 5′-TGTCT AGCTTATGAGCTTAGGG-3′. The PCR conditions were as follows: initial denaturing at 95°C for 10 min; 35 cycles at 95°C for 1 min, 52°C for 45 s, 72°C for 1 min and a final extension at 72°C for 5 min.

The resulting PCR products were genotyped by single base extension (SBE) (Juffer et al. 2009). The primers used for A55T, E164K, I225T K153R and P198A were 5′CTAAATCTTCAAGAATAGAA-3′, 5′-CAAACACTGTTGTAGGAGTCT-3′, 5′-CTG AATCCAACTTAGGCA-3′, 5′-TTTAATACAATA CAATAAAGTAGTAA-3′, and 5′-TTTTTTTT ATCTCTGAAACTTGACATGAAC-3′, respectively. The PCR SBE conditions were 96°C for 10 s; 25 cycles at 50°C for 5 s and 60°C for 30 s. The resulting PCR products were detected in an ABI PRISM (Applied Biosystems, Foster City, CA).

Results and discussion

Genotype success was 100%. No participant carried the mutant allele of any of the A55T, E164K, I225T and P198A variants, whereas three participants (all women) carried the R allele of the K153R polymorphism (total allelic frequency=4.9%), with one of them (woman aged 96 years) being homozygous. This is the first RR genotype that we have found for the *GDF8* K153R polymorphism in our entire DNA-base of Spanish population (~500 samples). To our knowledge, there is no available information regarding the muscle strength phenotypes of the few RR Caucasians that have been reported to exist. Corsi et al. (2002) found an old Italian person (assumed age <80 years) with the RR genotype, yet with no specification on individual phenotype data.

Figures 1 and 2 show the individual values of muscle strength and functional capacity phenotypes respectively. Overall, in the two KR women (labelled as # 1 and # 2 in Figs. 1 and 2) muscle phenotype values were low-to-normal compared to the whole group (~25th–50th sex-specific percentile) and their functional capacity during daily living was normal. In the woman with the RR genotype, values of muscle mass and functional capacity were below the 25th sex-specific percentile. To note was her almost null gait and balance ability (Tinetti scale) and her low capacity for performing ADLs independently (Barthel score) (Fig. 2).

The results should be taken as preliminary due to the small sample size of our cohort, yet we believe this is justifiable owing to the uniqueness of the studied population group and the low frequency of the R allele among Caucasians. Overall, our data suggest that, except in the few rare cases of homozygosity, the R allele of the *GDF8* K153R polymorphism is not associated with accelerated sarcopenia in humans who are near the end of the lifespan. Recent research from our laboratory showed that heterozygosity for this variation can alter the functional capacity of adult women, yet their muscle function was already altered by an inherited myopathy (Gonzalez-Freire et al. 2009). Although more research is needed, the *GDF8* K153R polymorphism has the potential to alter the function of the *GDF8* gene (Ferrell et al. 1999; Saunders et al. 2006), as briefly explained below. Myostatin enters the bloodstream as a latent precursor protein and then undergoes a proteolytic process to

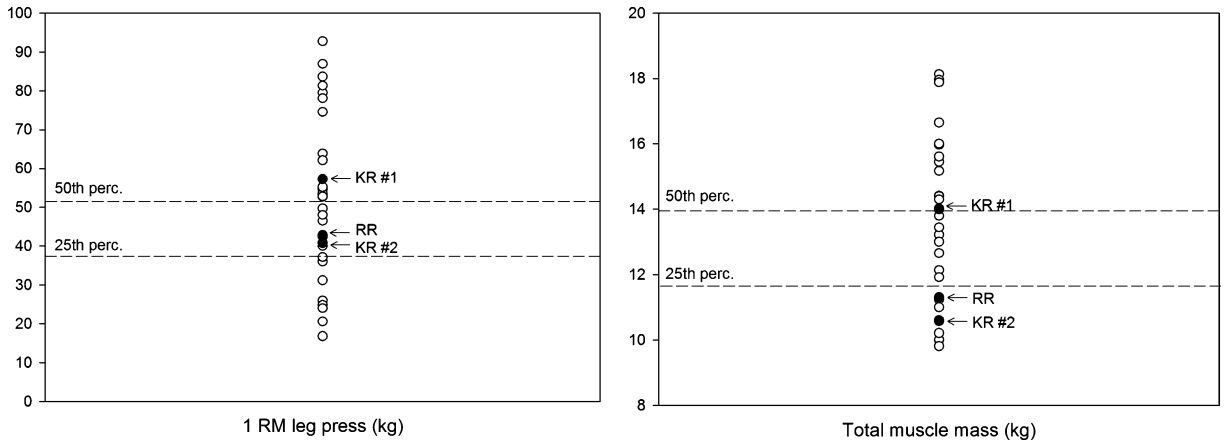

Fig. 1 Individual data of muscle phenotypes in all women. Abbreviations: perc. (sex-specific percentile)

become a mature peptide (free from the propeptide) that binds to extracellular activin type II receptor (ActRIIB) (Kostek et al. 2009). Binding of myostatin to ActRIIB induces intracellular activation of Smad proteins; through this pathway, myostatin modulates myoblast proliferation (Thomas et al. 2000) and differentiation (Ríos et al. 2002), and thus ultimately muscle mass. The Lys(K)153Arg(R) amino acid replacement is found within the active mature peptide of the myostatin protein and could theoretically influence (1) proteolytic processing with its propeptide or (2) affinity to bind with ActRIIB (Lee and McPherron 2001; Jiang et al. 2004). This in turn would result in inability of myostatin to modulate muscle mass and strength (Kostek et al. 2009).

In summary, and while keeping in mind that more research with larger population samples is necessary, heterozygosity for the *GDF-8* K153R polymorphism does not seem to exert a negative influence on the muscle strength and functional capacity of women who are at the end of the human lifespan, whereas homozygosity seems to impair these phenotypes. The RR genotype might explain some 'extreme' sarcopenia phenotypes among old humans. It must be however emphasised that phenotype traits indicative of muscle function in the elderly are probably complex and thus not likely to be reducible to the influence of a few single polymorphisms.

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Conflict of interest None

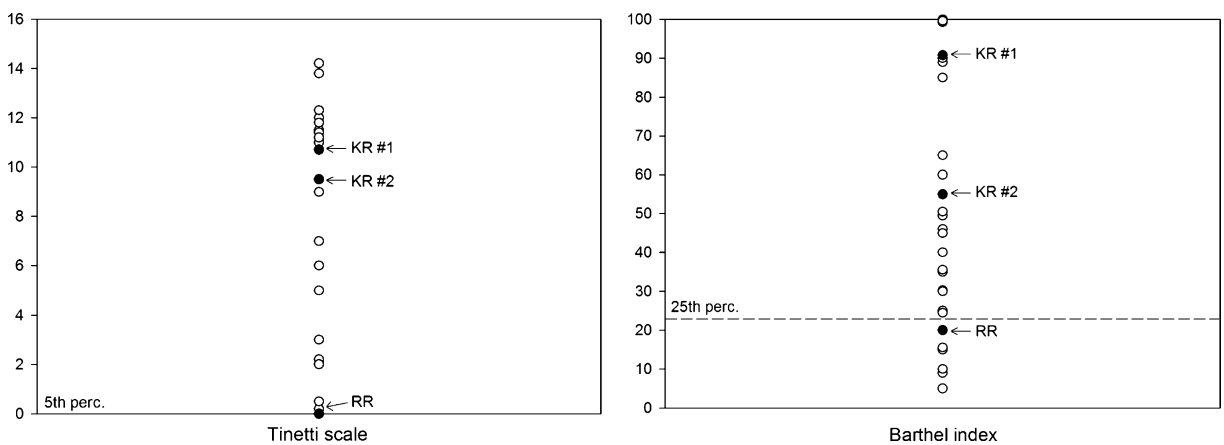

Fig. 2 Individual data of functional capacity tests. Abbreviations: perc. (sex-specific percentile)

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